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Effects of fiber or probiotic yogurt supplementation on intestinal barrier integrity in constipation-predominant irritable bowel syndrome

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ABSTRACT

Aims: This study aimed to evaluate the effects of different dietary treatments on intestinal integrity in female subjects aged 19-50 years with a previous diagnosis of constipation-predominant irritable bowel syndrome (IBS).

Methods: This randomized controlled trial was conducted at the Gastroenterology Clinic of Gülhane Training and Research Hospital, Ankara, Türkiye. Individuals with IBS were randomly assigned to three groups. Group 1 received a regular constipation diet (n=21), group 2 received a constipation diet rich in soluble fiber (n=17), and group 3 received a constipation diet supplemented with probiotic yogurt (n=22). All participants were followed up for 8 weeks. Intestinal integrity was assessed using plasma zonulin levels before and after treatment.

Results: The study included 60 patients (age, mean±SD 38.3±8.1 years). Following the intervention, zonulin levels showed non-significant increases from 24.41±25.10 to 28.59±24.05' (p=0.434) in group 1 and 25.91±25.10 to 28.59 (p=0.758) in group 2. It showed a non-significant decrease from 26.37±24.22 to 24.44±22.22 (p=0.393) in group 3. Fasting blood glucose, C-reactive protein, total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels also showed no significant differences between the groups at the beginning and end of the study. There was no significant relationship between zonulin levels and nutrient levels in group 1 and group 3 at the 8th-week measurements. In group 2, zonulin level was inversely and moderately correlated with fat percentage, monounsaturated fatty acid content, and vitamin E content (p<0.05). There was a linear, moderate relationship between zonulin levels and omega 6/omega 3 ratio (r=0.582; p=0.015).

Conclusions: The serum zonulin levels did not change significantly after consumption of fiber or probiotic yogurt (NCT06421922).

Introduction

Irritable bowel syndrome (IBS) is an intestinal disease characterized by abdominal pain, constipation, and/or diarrhea. IBS is a frequent disease with a 5-20% worldwide prevalence. In Western countries, its prevalence is 8-23%, of which 60-70% are women (1). In Türkiye, 10-14.9% of adults were found to have IBS, which was more frequent among women between the ages of 20-40 years (2).

Factors such as heredity, environment, diet, gastrointestinal microbiota, and inflammation in the gastrointestinal tract play a role in the pathogenesis of IBS (3). Hypersensitivity to certain nutrients may also contribute to pathogenesis by causing low grade intestinal inflammation and increased epithelial barrier permeability (4).

The epithelial cells in the intestinal mucosa are held together by tight bands tight junction (TJ) (5). TJ areas close the spaces between cells and form an intestinal barrier. In dysbiosis, bacterial toxins and lipopolysaccharides damage the intestinal mucosa and disrupt the function of the intestinal microbiota. With these stimuli, zonulin release from TJ points increases the permeability in the intestines (5). Increased intestinal permeability is thought to be an early stimulus leading to low-grade inflammation in the intestinal mucosa (6).

Serum zonulin levels increase in patients diagnosed with IBS (7). It has also been shown that zonulin may be a useful biomarker for altered intestinal permeability in patients with IBS (8).

In recent years, more attention has been paid to the role of diet in IBS (9). Dietary changes and nutritional habits differ among individuals, which significantly affect strategies for improving health and preventing diseases. To prevent IBS attacks, approaches such as increasing soluble fiber intake, eliminating foods thought to cause symptoms, and using probiotics/prebiotics are recommended in medical nutritional therapy (10).

Probiotics stabilize the intestinal microbiota and maintain its balance. Moreover, they increase mucosal integrity and improve the intestinal barrier (11). A meta-analysis concluded that the use of probiotics can reduce IBS symptoms (12). Soluble fiber also dissolves in water and forms consistency in the small intestine, showing little laxative effects because of its rapid fermentation (13). A meta-analysis of fourteen randomized controlled trials concluded that soluble fibers such as psyllium may favorably affect IBS courses (14). Therefore, this study evaluated the effects of different dietary treatments on zonulin levels in female subjects aged 19-50 years with a previous diagnosis of constipation-predominant IBS.

Methods

Study design and participants

This non-pharmacological randomized controlled study was conducted in the Gastroenterology Outpatient Clinic of Gülhane Training and Research Hospital, Ankara, Türkiye, between June 2019 and March 2020. The participants were women aged 19-50 years who were diagnosed with IBS. The inclusion criteria diagnosis with IBS according to the Rome 4 criteria 2017 (15), no metabolic disease history (e.g., diabetes mellitus, cardiovascular disease), no history of chronic disease such as cancer and autoimmune diseases, no use of probiotics, and no use of nutritional supplements (vitamins, minerals) in the last 6 months. The main exclusion criterion was pregnancy. This study followed the Helsinki Declaration guidelines and was registered at www.clinicaltrials.gov (NCT06421922).

Ethical approval for the study was obtained from the University of Health Sciences Türkiye, Gülhane Training and Research Hospital Non-Interventional Research Ethics Committee (ethics approval code: 46418926, project/decision no: 18/253, evaluation date: 21.11.2018). All participants signed a voluntary consent form, and participation in the study was voluntary.

Dietary randomization

The participants were randomly assigned to three groups using random allocation software for parallel group randomized trials (16). Group 1 received a regular constipation diet; group 2 received a constipation diet rich in soluble fiber, and group 3 received a constipation diet supplemented with probiotic yogurt. The constipation diet included 2 L of water, 2 portions of vegetables, and 3 portions of fruits and legumes 2 times a week. Soluble fiber (resistant starch) (5 g/day) was added to the constipation diet in group 2 as 1 sachet/day (5 g/day) during the initial 4 weeks and 2 sachets/day (10 g/day) during the subsequent 4 weeks. “*Bifidobacterium infantis 35624 (B. infantis 35624)*” strain, specific to IBS, was added to yogurt in group 3 and consumed before lunch. The follow-up period was 8 weeks.

All data were collected via face-to-face surveys. In the first visit, we assessed sociodemographic characteristics, 3-day food consumption, serum zonulin level, and biochemical tests [fasting blood glucose, cholesterol, blood triglyceride, low density lipoprotein (LDL) cholesterol, and C-reactive protein (CRP)]. Serum zonulin levels were measured using a “BT Lab Human zonulin ELISA Kit” (China, E1117). Three-day food consumption was recorded for 2 consecutive days on weekdays and one day on weekends. The daily energy and nutrients intake were analyzed using the Nutrition Information System 8 (BeBis 8) computer package program (17). Biochemical tests and serum zonulin levels were measured at the beginning of the study and at the end of the 8th week.

Power and sample size

The sample size was calculated using G*Power (G*Power Ver. 3.1.9.7, Franz Faul, Universität Kiel, Germany). With an estimated 90% power, α=0.05 type 1 error, β=0.10 type 2 error, and f=0.25 effect size, the required total sample size was 54, comprising 18 participants in each group. To compensate for the exclusions, 10% more patients were enrolled, resulting in 60 participants. A total of 100 patients were initially invited, but 10 were excluded because they did not fulfill the inclusion criteria. Finally, 31 volunteers were assigned to groups 1, 30 to groups 2, and 29 to groups 3. A total of 30 volunteers (10 in group 1, 13 in group 2, and 7 in group 3) were excluded from the study because they did not participate in the control visits.

Primary and secondary outcomes

The primary outcome was the change in blood zonulin levels after the addition of probiotic yogurt to a regular constipation diet instead of soluble fiber. The secondary outcomes were changes in fasting blood glucose, cholesterol, blood triglyceride, LDL cholesterol, and CRP levels after adding probiotic yogurt to a regular constipation diet instead of soluble fiber.

Statistical Analysis

The obtained data were analyzed using IBM Statistical Package for the Social Sciences (SPSS) Statistics for Windows, version 22.00 (IBM Corp., Armonk, NY, USA). The normality of continuous variables (age, zonulin level, fasting blood sugar, CRP, total and LDL cholesterol, triglycerides) was assessed using the Shapiro Wilk test. To make continuous variables more understandable and ensure consistency with findings from other studies, they are presented as mean±standard deviation (SD). Correlations between continuous variables are displayed using Spearman rank correlation coefficients. Inter-group comparisons were made by analysis of variance or Kruskal-Wallis test, as appropriate. Baseline to 8th-week within-group comparisons were performed using the Wilcoxon signed-rank test. The significance level was set at $p<0.05$.

Results

The study included 60 patients with a mean age of 38.3±8.1 years. Groups 1, 2, and 3 consisted of 21, 17, and 22 patients, respectively.

Biochemical findings

Compared with the baseline, there was no change in zonulin level at 8th week in any group, despite some increases of approximately 3 ng/mL in groups 1 and 2, and a decrease of approximately 2 ng/mL in group 3 (Table 1). There were also no intergroup differences in zonulin levels between baseline and follow-up.

At baseline and 8th week, biochemical parameters including fasting blood glucose, total cholesterol, LDL cholesterol, triglyceride, and CRP were similar in the three groups (Table 1). Similarly, there was no change from baseline to the 8th week in the levels of these biochemical parameters (Table 1).

Energy and nutrient intake

As shown in Table 2, dietary energy intake decreased in groups 1 and 2 and remained unchanged in group 3 by the 8th week. In all three groups, proteins comprised approximately 16-17% of the energy intake at baseline and 8th week. While the percentage of energy intake from carbohydrates was approximately 40% at baseline, it decreased to 37-38% at the 8th week. The percentage of energy intake from fat increased significantly in group 1 during the study period (p=0.021), whereas the increase in the other groups was not statistically significant (p>0.05) (Table 2).

Table 1. Biochemical findings of individuals in different dietary treatment groups

	Group 1 (n=21)			Group 2 (n=17)			Group 3 (n=22)			Inter-groups	
	Baseline	**8th week**		**Baseline**	**8th week**		**Baseline**	**8th week**		**Baseline**	**8th week**
Biochemical findings	$\bar{x}$±SD	$\bar{x}$±SD	p^{1**}	$\bar{x}$±SD	$\bar{x}$±SD	p^{2**}	$\bar{x}$±SD	$\bar{x}$±SD	p^{3**}	p^{4*}	p^{5*}
Fasting blood glucose (mg/dL)	91.33±8.92	89.67±13.04	0.596	90.88±24.68	91.18±12.95	0.933	93.39±18.84	91.64±18.96	0.574	0.898	0.911
Cholesterol (mg/dL)	181.00±25.74	174.85±26.14	0.352	184.76±34.26	199.76±48.43	0.434	178.00±39.35	179.45±37.92	0.821	0.824	0.115
Triglyceride (mg/dL)	105.48±75.30	101.14±64.4	0.362	154.65±141.68	137.59±98.82	0.469	88.55±31.60	90.05±29.62	0.884	0.073	0.126
LDL-C (mg/dL)	108.00±18.54	101.86±17.92	0.054	111.71±27.48	126.88±42.88	0.163	113.18±40.01	110.36±30.45	0.626	0.731	0.173
CRP (mg/L)	3.33±5.31	4.81±10.12	0.274	1.75±1.85	2.11±3.07	0.535	1.82±2.39	2.18±2.63	0.715	0.449	0.809
Zonulin (ng/mL)	25.41±25.10	28.59±24.05	0.434	25.91±25.10	28.59±24.05	0.758	26.37±24.22	24.44±22.22	0.393	0.923	0.893

*Wilcoxon test for within-group comparisons or analysis of variance for repeated measures.
**Mann-Whitney U test for intergroup comparisons or analysis of variance for repeated measurements, $p<0.05$. p^1: comparison of the before and after values of the 1st group, p^2: comparison of the before and after values of the 2nd group, p^3: comparison of the before and after values of the 3rd group, p^4: comparison of the initial values of the groups, p^5: comparison of the 8th week values of the groups.
SD: Standard deviation, LDL-C: Low-density lipoprotein-cholesterol, CRP: C-reactive protein

Table 2. Energy and nutrient intake of individuals in different dietary treatment groups

	Group 1			Group 2			Group 3			Overall	
	Baseline	8th week		Baseline	8th week		Baseline	8th week		Baseline	8th week
Energy and nutrients	**$\bar{x}$±SD**	**$\bar{x}$±SD**	**p^1**	**$\bar{x}$±SD**	**$\bar{x}$±SD**	**p^2**	**$\bar{x}$±SD**	**$\bar{x}$±SD**	**p^3**	**p^4**	**p^5**
Energy (kcal/day)	1546.36±681.64	1262.94±232.41	**0.021**	1274.04±439.97	1099.01±152.52	0.192	1445.49±526.28	1456.16±476.84	0.927	0.340	**0.006**[c]
Carbohydrate (g/day)	149.66±67.5	115.56±30.68	**0.014**	125.56±49.72	100.53±18.66	0.072	141.15±48.38	138.07±61.0	0.798	0.422	**0.026**[c]
Carbohydrate (%)	40.05±6.87	37.10±5.05	0.125	40.12±5.78	37.59±7.62	0.231	40.5±5.73	38.09±5.04	0.195	0.967	0.858
Simple carbohydrates (%)	22.72±16.61	17.05±4.38	**0.011**	7.89±11.72	4.46±6.54	0.598	23.5±34.52	25.0±30.0	0.836	0.068	**0.001**[a]
Protein (g/day)	64.68±27.6	51.28±9.97	**0.025**	50.39±12.87	47.78±10.72	0.674	60.84±26.27	59.48±22.89	0.800	0.180	0.071
Protein (%)	17.52±3.8	16.76±3.37	0.474	16.76±3.21	17.82±3.13	0.366	16.86±2.85	16.59±2.68	0.789	0.733	0.423
Plant-based protein (g/day)	21.77±10.66	18.56±4.44	0.163	18.06±6.76	16.21±3.53	0.398	18.52±7.25	20.19±7.78	0.371	0.326	0.107
Animal protein (g/day)	42.91±24.34	32.72±9.19	**0.048**	32.33±10.64	31.56±11.76	0.887	42.32±19.72	39.29±16.9	0.514	0.195	0.138
Fat (g/day)	74.81±38.32	65.51±13.8	0.199	62.63±25.0	55.5±12.61	0.362	69.75±28.65	72.49±19.05	0.687	0.500	**0.005**[c]
Fat (%)	42.19±5.23	46.05±4.38	**0.021**	43.12±5.95	44.41±6.25	0.454	42.64±5.08	45.27±4.94	0.086	0.870	0.626
SFA (g/day)	25.37±13.54	20.42±5.03	0.064	18.25±6.02	17.91±3.34	0.945	24.15±12.08	23.59±9.54	0.821	0.136	**0.038**[c]
SFA (%)	14.63±3.50	14.53±1.70	0.982	13.11±2.83	14.68±2.25	0.276	14.63±3.50	14.53±1.70	0.922	0.230	0.900
MUFA (g/day)	23.88±11.96	21.17±3.13	0.236	20.84±9.3	18.17±2.91	0.291	22.75±9.89	15.89±5.18	0.793	0.674	**0.021**[c]
MUFA (%)	13.68±1.99	15.14±1.55	0.055	14.46±3.38	14.77±1.98	0.862	13.68±1.99	15.14±1.55	0.364	0.630	0.440
PUFA (g/day)	20.67±13.58	19.11±5.97	0.574	19.59±12.05	15.82±5.89	0.224	17.95±7.29	20.73±4.89	0.304	0.726	**0.028**[c]
PUFA (%)	11.69±3.91	13.65±3.60	0.687	13.32±3.70	12.68±3.79	0.383	11.69±3.91	13.65±3.60	0.118	0.200	0.670
Omega-6/omega-3	0.08±0.05	0.06±0.02	0.129	0.05±0.02	0.06±0.03	0.480	0.08±0.06	0.06±0.03	**0.027**	0.083	0.936
Cholesterol (mg/day)	263.66±190.20	253.39±83.32	0.765	197.6±90.17	222.72±84.21	0.515	286.55±157.21	305.26±149.41	0.573	0.202	0.075
Fiber (g/day)	15.69±6.45	18.35±1.54	0.582	15.12±4.88	25.78±1.66	0.827	14.59±5.05	15.89±2.06	0.264	0.808	0.474
Soluble fiber (g/day)	5.15±2.22	5.13±1.84	0.963	4.51±2.19	14.52±2.0	0.918	4.29±1.98	4.74±3.50	0.319	0.393	0.503
Insoluble fiber (g/day)	9.5±3.96	9.95±7.45	0.534	8.38±2.68	8.80±4.04	0.595	8.54±2.65	10.12±7.45	0.251	0.483	0.259
Vitamin A (mcg/day)	1097.25±1135.35	740.64±198.38	0.212	834.29±471.21	677.55±238.53	0.624	793.03±451.37	1145.71±1649.59	0.202	0.388	0.284

Table 2. Continued

	Group 1			Group 2			Group 3			Overall	
	Baseline	**8th week**		**Baseline**	**8th week**		**Baseline**	**8th week**		**Baseline**	**8th week**
Energy and nutrients	**x̄±SD**	**x̄±SD**	**p^1**	**x̄±SD**	**x̄±SD**	**p^2**	**x̄±SD**	**x̄±SD**	**p^3**	**p^4**	**p^5**
Vitamin C (mg/day)	62.19±39.60	71.95±33.60	0.255	65.4±33.92	68.7±27.78	0.941	64.51±30.84	63.92±38.82	0.943	0.817	0.741
Vitamin E (mg/day)	19.42±12.66	18.63±5.61	0.776	19.89±12.19	16.26±5.85	0.223	16.33±6.80	20.86±5.34	0.085	0.517	**0.045^c**
Vitamin K (mcg/day)	308.3±172.63	307.23±61.79	0.977	282.91±116.93	265.22±59.16	0.631	249.89±130.85	292.6±121.29	0.188	0.414	0.344
Thiamine (mg/day)	0.63±0.24	0.56±0.09	0.148	0.54±0.15	0.55±0.12	0.842	0.59±0.19	0.62±0.20	0.491	0.390	0.243
Riboflavin (mg/day)	1.08±0.50	0.89±0.12	0.099	0.90±0.22	0.86±0.14	0.766	1.08±0.43	1.17±0.53	0.407	0.294	**0.008^c**
Niacin (mg/day)	21.50±9.24	16.62±3.96	**0.021**	16.39±4.89	15.8±4.40	0.792	19.54±8.0	20.48±8.20	0.633	0.138	**0.034^c**
Vitamin B_6 (mg/day)	1.07±0.48	0.83±0.10	**0.012**	0.85±0.23	0.78±0.13	0.475	0.99±0.37	0.95±0.39	0.617	0.234	0.114
Folic acid (mcg/day)	205.44±85.02	211.03±38.15	0.746	203.92±64.02	198.69±34.09	0.784	217.49±84.53	235.11±81.20	0.279	0.836	0.133
Vitamin B_{12} (μg/day)	4.15±2.79	3.10±0.64	0.315	2.83±1.25	2.86±0.81	0.985	4.57±3.88	4.94±6.01	0.719	0.183	0.151
Iron (mg/day)	9.25±3.95	8.23±1.47	0.178	8.05±2.21	7.50±1.12	0.532	8.82±3.41	8.82±2.79	0.995	0.543	0.131
Magnesium (mg/day)	204.45±84.43	186.37±32.44	0.310	168.69±40.99	174.34±42.67	0.771	189.44±82.95	203.43±64.98	0.416	0.343	0.188
Zinc (mg/day)	8.47±3.75	7.29±1.15	0.122	6.59±1.48	6.70±1.34	0.894	8.34±3.82	8.18±2.83	0.817	0.168	0.070
Calcium (mg/day)	563.82±284.52	489.16±100.55	0.191	466.25±114.22	453.84±100.71	0.847	522.80±259.74	597.39±254.25	0.18	0.460	**0.030^c**
Potassium (mg/day)	1710.66±742.91	1506.17±201.93	0.155	1480.87±395.43	1403.92±245.76	0.638	1658.41±599.48	1682.77±663.95	0.861	0.492	0.140
Phosphorus (mg/day)	921.85±372.58	805.5±122.32	0.136	752.81±161.95	750.98±116.82	0.986	895.13±378.21	940.96±307.16	0.543	0.258	**0.017^c**
Cupper (mg/day)	1.17±0.49	1.03±0.14	0.148	1.06±0.32	0.95±0.14	0.342	1.13±0.39	1.20±0.46	0.458	0.702	**0.034^c**

The fiber content in the second group: 5 g in the first 4 weeks and 10 g in the second 4 weeks.
*Analysis of variance in group comparisons and repeated measurements. **Analysis of variance in group comparisons, repeated measurements, Bonferroni test in pairwise comparisons, $p<0.005$. p^1: before and after the first group, p^2: before and after the second group, p^3: before and after the third group, p^4: comparison of the initial values of the groups, p^5: comparison of the 8th week values of the groups. ^{a}: difference between 1st and 2nd groups, ^{b}: difference between 1st and 3rd groups, ^{c}: a difference between 2nd and 3rd groups.
SD: Standard deviation, SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid

Relationship between serum zonulin levels and nutrient intake

At baseline, plant-based protein (r=-0.565; p=0.008) and soluble fiber (r=-0.626; p=0.002) were inversely correlated with zonulin levels in group 1. There was also a moderate linear relationship between cholesterol and zonulin levels (r=0.440; p=0.046). In group 2, there was a linear correlation between zonulin levels and protein intake (r=0.485; p=0.049). In group 3, there was an inverse correlation between the levels of zonulin and Monounsaturated fatty acid (MUFA) (r=-0.501; p=0.018).

There was no significant correlation between zonulin levels and nutrient intake in groups 1 and group 3 by the 8th week. In group 2, the zonulin level was inversely correlated with the percentage of fat (r=-0.549; p=0.022), MUFA (r=-0.547; p=0.023) and Vitamin E (r=-0.525; p=0.031). There was a positive correlation between levels of zonulin and omega 6/omega 3 ratio (r=0.582; p=0.015) (Table 3).

Discussion

This study was planned and conducted to evaluate the effects of different dietary treatments on several biochemical parameters [fasting blood glucose, CRP, cholesterol (total and LDL), triglyceride] and zonulin levels in female subjects aged 19-50 years with a previous diagnosis of IBS.

The diagnosis of IBS is a “symptom-based” disease. Elevated CRP level is also an important symptom of IBS (18). Although considered a functional disorder, intestinal inflammation is an element of the pathophysiology of IBS. Therefore, plasma high-sensitivity CRP, a marker of micro-inflammation, may be elevated in IBS (19).

Dietary fiber has anti-inflammatory effects by reducing lipid oxidation (20). Conversely, a low-fiber diet increases the levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6), IL-18, and tumor necrosis factor-alpha (21). An epidemiological study showed that increased dietary fiber intake was significantly associated with lower CRP levels (22). Several authors have also reported reduced serum IL-6, CRP, C-peptide, and insulin levels following higher consumption of whole grain products (23). In mouse models of colorectal cancer, consumption of resistant starch increases the production of short-chain fatty acids and reduces inflammation and cell proliferation (24).

Probiotic supplementation increases immunity, reduces inflammation by stimulating cytokines that prevent inflammation, and prevents the growth of pathogens (25). In addition, probiotics affect immune cells and stimulate the production and secretion of anti-inflammatory cytokines (26). In a double-blind, placebo controlled study by Hod et al. (19), after 8 weeks of probiotic supplementation in individuals with diarrhea-predominant IBS, CRP levels did not significantly change compared with baseline. In another study, total and LDL cholesterol levels were reduced following supplementation with probiotics among individuals with obesity (27). However, at the end of the study, the observed difference was not statistically significant despite the positive effects of probiotics on lipid parameters (27).

No difference was observed in the levels of biochemical parameters at baseline or at the end of our study. The lack of a decrease in CRP levels after 8 weeks in the soluble fiber and probiotic supplement groups may be related to factors such as stress since CRP is an indicator of acute inflammation. The lack of a decrease in biochemical parameters in the Infantis 35624 supplement group may be due to the higher saturated fat consumption of individuals in that group.

Dietary fiber has a positive effect on both inflammation and intestinal permeability. With high fiber intake, the number of bacteria that produce short-chain fatty acids in the intestine increases. Short-chain fatty acids help reduce inflammation by promoting intestinal tissue repair and increasing mucus secretion (28). This study showed that both constipation predominant and diarrhea-predominant IBS zonulin levels were higher than in the control group. Zonulin may be a useful simple biomarker for altered intestinal permeability in patients with IBS (8). In another study, supplementation with kefir, a local product rich in probiotics, for 3 weeks improved serum zonulin levels compared with milk supplementation among overweight subjects (29). Obese individuals who received frozen green leafy vegetables during the first or last four weeks of a 12-week trial had increased serum zonulin levels with no effect on fecal zonulin levels (30). Significant reductions in serum zonulin levels were also observed in IBS patients who received probiotic therapy for 12 weeks, but not in those treated for 8 weeks (31).

In the present study, serum zonulin levels did not increase in the intervention groups. The reason supplementation with fiber or probiotics did not affect zonulin levels may be related to the higher percentage of dietary fat intake than the recommended value by TÜBER as 2015 recommendations include 25-30% of energy from fat sources (32). Animal studies have shown that a high-fat diet increases intestinal permeability and decreases the expression of TJ proteins such as zonulin and occludin in intestinal epithelial cells, thereby accelerating the passage of bacterial endotoxins into the blood (20). In humans, data are sparse because serum zonulin levels are correlated with fat intake only in several studies (33-36). However, there are notable differences between the published studies regarding participant characteristics and study design.

We observed that the serum zonulin levels of individuals in the first group were negatively correlated with the amount of dietary plant-based protein and soluble fiber and positively correlated with cholesterol. This finding may be due to the anti-inflammatory and intestinal barrier-strengthening effects of butyrate, an end-product of the fermentation of soluble fiber (37). The positive correlation between zonulin levels and cholesterol levels in group 1 may be due to high-fat consumption

Table 3. **Relationship between pre- and posttreatment zonulin levels and energy and nutrient intake levels among individuals receiving different dietary treatments**

Energy and nutrients	Zonulin level (ng/mL) (baseline)						Zonulin level (ng/mL) (8th week)					
	Group 1 (n=21)		Group 2 (n=17)		Group 3 (n=22)		Group 1 (n=21)		Group 2 (n=17)		Group 3 (n=22)	
	r	p	r	p	r	p	r	p	r	p	r	p
Energy (kcal/day)	-0.312	0.169	-0.223	0.390	-0.322	0.143	0.248	0.278	-0.087	0.740	0.065	0.774
Carbohydrate (g/day)	-0.373	0.096	-0.265	0.305	-0.322	0.143	0.113	0.626	0.418	0.095	0.033	0.883
Carbohydrate (%)	-0.317	0.161	0.009	0.974	0.210	0.348	0.134	0.564	0.417	0.096	-0.041	0.855
Protein (g/day)	-0.047	0.841	-0.110	0.673	-0.302	0.172	0.099	0.670	-0.092	0.726	-0.054	0.813
Protein (%)	0.289	0.204	0.485	**0.049**	-0.145	0.519	-0.073	0.754	0.088	0.736	-0.083	0.714
Plant-based protein (g/day)	-0.565	**0.008**	-0.203	0.434	-0.351	0.110	-0.021	0.929	0.210	0.419	0.028	0.903
Animal-based protein (g/day)	0.082	0.724	0.174	0.504	-0.330	0.133	0.144	0.533	-0.085	0.745	-0.015	0.946
Fat (g/day)	-0.149	0.518	-0.272	0.291	-0.261	0.240	0.182	0.430	-0.460	0.063	0.091	0.687
Fat (%)	0.199	0.387	-0.251	0.331	-0.206	0.357	0.088	0.706	-0.549	**0.022**	0.066	0.769
Saturated fatty acid (g/day)	0.430	0.052	0.235	0.363	-0.322	0.143	-0.008	0.973	-0.131	0.616	-0.234	0.294
MUFA (g/day)	0.312	0.169	-0.250	0.333	-0.501	**0.018**	0.277	0.225	-0.547	**0.023**	-0.014	0.950
PUFA (g/day)	-0.384	0.085	-0.208	0.422	-0.072	0.751	0.061	0.793	-0.635	0.006	0.178	0.428
Omega-6/omega-3	0.340	0.131	0.012	0.963	-0.411	0.058	-0.173	0.454	0.582	**0.015**	0.128	0.570
Cholesterol (mg/day)	0.440	**0.046**	-0.211	0.417	-0.119	0.597	0.218	0.342	-0.088	0.736	0.086	0.702
Fiber (g/day)	-0.410	0.065	-0.147	0.573	-0.407	0.060	-0.090	0.699	-0.012	0.963	-0.179	0.425
Soluble fiber (g/day)	-0.626	**0.002**	-0.150	0.567	-0.341	0.120	-0.117	0.614	0.098	0.708	-0.380	0.081
Insoluble fiber (g/day)	-0.426	0.054	-0.007	0.978	-0.324	0.142	-0.110	0.634	0.115	0.659	-0.083	0.713
Vitamin A (mcg/day)	0.210	0.360	-0.145	0.580	0.001	0.998	-0.036	0.876	-0.056	0.830	0.084	0.710
Vitamin C (mg/day)	-0.023	0.920	-0.473	0.055	0.126	0.577	-0.034	0.884	-0.395	0.117	0.190	0.396
Vitamin E (mg/day)	-0.423	0.056	-0.301	0.240	0.057	0.801	0.042	0.858	-0.525	**0.031**	0.156	0.487
Vitamin K (mcg/day)	-0.174	0.451	-0.368	0.147	-0.235	0.291	-0.208	0.366	-0.298	0.245	0.077	0.732
Niacin (mg/day)	0.000	1.000	0.022	0.933	-0.319	0.148	0.131	0.571	-0.092	0.725	0.042	0.852
Folic acid (mcg/day)	-0.287	0.208	-0.229	0.376	-0.391	0.072	-0.051	0.827	0.071	0.786	-0.020	0.930
Vitamin B$_{12}$ (µg/day)	-0.342	0.130	-0.225	0.384	-0.182	0.417	0.032	0.889	-0.170	0.513	-0.121	0.590
Iron (mg/day)	0.282	0.216	0.001	0.996	-0.068	0.763	0.130	0.575	0.002	0.993	0.040	0.859
Magnesium (mg/day)	-0.279	0.220	-0.262	0.309	-0.331	0.132	-0.161	0.486	0.077	0.768	0.158	0.484
Zinc (mg/day)	-0.294	0.197	-0.042	0.874	-0.418	0.053	-0.148	0.522	-0.244	0.345	-0.092	0.684
Calcium (mg/day)	-0.184	0.423	0.094	0.719	-0.267	0.230	-0.095	0.683	0.056	0.830	0.010	0.966
Potassium (mg/day)	0.039	0.867	0.051	0.844	-0.153	0.497	0.114	0.622	-0.168	0.519	-0.061	0.787
Sodium (mg/day)	-0.206	0.369	-0.238	0.358	-0.348	0.112	0.071	0.758	0.023	0.929	-0.167	0.459
Phosphorus (mg/day)	-0.109	0.638	-0.115	0.660	-0.252	0.257	0.123	0.594	-0.195	0.453	0.063	0.782
Cupper (mg/day)	-0.094	0.687	-0.105	0.687	-0.240	0.282	0.197	0.391	-0.078	0.765	-0.043	0.848

r: Spearman rank correlation coefficient, $p<0.05$.
MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid

in this group, as a high-fat meal can cause inflammation and the formation of advanced glycation end products associated with increased oxidative stress and inflammation (38).

Blood samples were not collected from certain patients at the conclusion of the investigation because their follow-up appointments occurred during the COVID-19 pandemic. This resulted in a smaller sample size than anticipated for the study.

Conclusion

The serum zonulin level did not change after fiber or probiotic yogurt supplementation. Future randomized controlled trials with larger sample sizes are needed to evaluate the effects of fiber and probiotic yogurt on serum zonulin levels in individuals with IBS.

Ethics

Ethics Committee Approval: Ethical approval for the study was obtained from the University of Health Sciences Türkiye, Gülhane Training and Research Hospital Non-Interventional Research Ethics Committee (ethics approval code: 46418926, project/decision no: 18/253, evaluation date: 21.11.2018).

Informed Consent: All participants signed a voluntary consent form.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.G., N.E.Ü., Concept: M.G., G.A., N.E.Ü., Design: G.A., N.E.Ü., Data Collection or Processing: N.E.Ü., Analysis or Interpretation: G.A., N.E.Ü., Literature Search: N.E.Ü., Writing: N.E.Ü.

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